DOCKET NO.: ISIS-5027 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Stanley T. Crooke

Serial No.: not yet assigned **Group Art Unit:** 1635

Filing Date: February 20, 2002 **Examiner:** S. McGarry

For: OLIGORIBONUCLEOTIDES AND RIBONUCLEASES FOR CLEAVING RNA

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Please make the following amendments to the above-identified application.

In the Sequence Listing:

Please delete the Sequence Listing on file and insert therefore pages **1-2** comprising the most recently filed Sequence Listing.

In the Claims:

Please cancel claim 1 without prejudice. Please add new claims **94-154**.

94 **(New)** A method of modification of a target RNA comprising contacting the target RNA with a double stranded RNA having first and second strands, said first strand comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside;

the first and second strands being hybridized to each other;

at least a portion of the first strand being complementary to the target RNA, said target RNA thereby being modified.

95. **(New)** The method of claim 94 wherein the first and second strands are covalently bound together.

96. **(New)** The method of claim 94 wherein the first and second strands form a single strand of RNA.

97. **(New)** The method of claim 94 wherein a plurality of target RNAs are modified.

98. **(New)** The method of claim 94 wherein said modification is characterized by cleavage of said target RNA.

99. **(New)** The method of claim 94 wherein said cleavage is in vitro.

100. **(New)** The method of claim 94 wherein the target RNA is present in a cell.

101. **(New)** The method of claim 100 wherein the target RNA is present in the cytoplasm of the cell.

102. **(New)** The method of claim 100 wherein the target RNA is present in the nucleus of the cell.

103. **(New)** The method of claim 100 wherein the target RNA is present in the mitochondria of the cell.

104. **(New)** A method of modification of a target RNA comprising contacting the target RNA with an oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside, said target RNA thereby being modified.

105. **(New)** A method of activating a nuclease activity within a cell comprising:
administering to the cell a double stranded RNA having first and second strands, said first strand comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside;

the first and second strands being hybridized to each other;

at least a portion of the first strand being complementary to an RNA in the cell,
said nuclease activity thereby being activated.

106. **(New)** The method of claim 105. wherein the first and second strands are covalently bound together.

107. **(New)** The method of claim 105 wherein the first and second strands form a single strand of RNA.

108. **(New)** The method of claim 105 wherein the nuclease activity is characterized by the production of at least one cleavage product, said cleavage product containing a 3' terminal hydroxyl moiety.

109. **(New)** The method of claim 105 wherein the nuclease activity is characterized by the production of at least one cleavage product, said cleavage product containing a 5' terminal phosphate moiety.

110. **(New)** The method of claim 109 wherein said cleavage product further contains a 3'

hydroxyl moiety.

111. **(New)** The method of claim 105 wherein the nuclease activity is cleavage by a dsRNAse enzyme.

112. **(New)** An isolated cleavage product generated by the method of any one of claims 108-110.

113. **(New)** A method of activating a nuclease activity within a cell comprising contacting the target RNA with an oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside, said nuclease activity thereby being activated.

114. **(New)** A method of forming a double stranded RNA in a cell comprising administering to said cell an oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; wherein the nucleic acid sequence of said oligomeric compound is complementary to and hybridizes with at least a four nucleobase portion of a target RNA in said cell, said double stranded RNA thereby being formed.

115. **(New)** The method of claim 114 wherein said oligomeric compound is from about 8 to about 50 nucleotides in length.

116. **(New)** The method of claim 114 wherein said oligomeric compound is from about 12 to about 30 nucleotides in length.

117. **(New)** The method of claim 114 wherein said target RNA comprises an mRNA.

118. **(New)** The method of claim 114 wherein said four nucleobase portion of said target RNA is within a coding region of said target RNA.

119. **(New)** The method of claim 114 wherein the double stranded RNA is formed in vitro.

120. **(New)** A double stranded RNA formed by the method of any one of claims 114-119.

121. **(New)** A method of modulating the level of one or more target RNA comprising contacting said target RNA with an oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside, said level of target RNA thereby being modulated.

122. **(New)** The method of claim 121 wherein said modulation is in vitro.

123. **(New)** A method of modulating the level of one or more target RNA comprising contacting the target RNA with a double stranded RNA having first and second strands, said first strand comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside;

the first and second strands being hybridized to each other;

at least a portion of the first strand being complementary to the target RNA, said level of target RNA thereby being modulated.

124. **(New)** A method of eliciting a dsRNAse response in a cell comprising administering to said cell an oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside, said dsRNAse response thereby being elicited.

125. **(New)** A method of eliciting a dsRNAse response in a cell comprising administering to said cell a double stranded RNA having first and second strands, said first strand comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside;

the first and second strands being hybridized to each other;

at least a portion of the first strand being complementary to the target RNA, said dsRNAse response thereby being elicited.

126. **(New)** The method of claim 124 or 125 wherein said dsRNase response is elicited in vitro.

127. **(New)** An oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside.

128. **(New)** The oligomeric compound of claim 127which is from about 12 to about 30 nucleosides in length.

129. **(New)** The oligomeric compound of claim 127 having a 3' terminal hydroxyl moiety.

130. **(New)** The oligomeric compound of claim 127 having a 5' terminal phosphate moiety.

131. **(New)** The oligomeric compound of claim 129 having a 5' terminal phosphate moiety.

132. **(New)** The oligomeric compound of claim 127 which is an oligonucleotide probe or primer.

133. **(New)** An oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to

modulate at least one of; binding affinity or binding specificity of said oligomeric compound.

134. **(New)** An oligomeric compound comprising at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside; said modified nucleoside adapted to modulate at least one of; pharmacokinetic property, absorption, distribution, clearance or charge of said oligomeric compound.

135. **(New)** An oligomeric compound comprising;

a first segment, said first segment comprising at least four consecutive 2'-hydroxyl ribonucleosides; and

a second segment, said second segment comprising at least one modified nucleoside; wherein said first and second segments are complementary to one another.

136. **(New)** The oligomeric compound of claim 135 wherein said first and second segments hybridize to one another to form a double stranded RNA, said double stranded RNA comprising said four consecutive 2'-hydroxy ribonucleosides.

137. **(New)** The oligomeric compound of claim 136 wherein a portion of said first segment and a portion of said second segment are complementary to one another and hybridize to one another to form a double stranded RNA.

138. **(New)** A double stranded RNA comprising the oligomeric compound of claim 127 hybridized to a complementary strand of RNA.

139. **(New)** The double stranded RNA of claim 138 formed inside a cell.

140. **(New)** The double stranded RNA of claim 138 wherein said complementary strand of

RNA is complementary to at least a four nucleobase portion of a target RNA.

141. **(New)** The double stranded RNA of claim 138 wherein the complementary strand of RNA comprises a target RNA.

142. **(New)** The double stranded RNA of claim 141 wherein the target RNA comprises a pre-mRNA transcript.

143. **(New)** The double stranded RNA of claim 141 wherein the target RNA comprises a mature mRNA transcript.

144. **(New)** The double stranded RNA of claim 138 wherein the target RNA is present in a cell's cytoplasm.

145. **(New)** The double stranded RNA of claim 138 wherein the target RNA is present in a cell's nucleus.

146. **(New)** The double stranded RNA of claim 138 wherein the target RNA is present in a cell's mitochondria.

147. **(New)** The double stranded RNA of claim 138 which is capable of effecting cleavage of at least one RNA.

148. **(New)** A pharmaceutical composition comprising the compound of claim 127 in a pharmaceutically acceptable diluent or carrier.

149. **(New)** A pharmaceutical composition comprising the compound of claim 138 in a

pharmaceutically acceptable diluent or carrier.

150. **(New)** A double stranded RNA comprising the oligomeric compound of claim 127 hybridized to a complementary strand of RNA, said double stranded RNA the substrate of a dsRNase.

151 **(New)** An assay device or kit for the reduction of one or more target RNA, said device or kit comprising the oligomeric compound of claim 127.

152. **(New)** An assay device or kit for the reduction of a target RNA, said device or kit comprising the double stranded RNA of claim 138.

153. **(New)** An assay device or kit for the detection of one or more target RNA comprising the oligomeric compound of claim 127.

154. **(New)** An assay device or kit for the detection of one or more target RNA comprising the double stranded RNA of claim 138.

REMARKS

Claim 1 was pending. Claim 1 has been cancelled without prejudice. New claims 94-154 have been added. No new matter has been added.

Upon entry of this amendment, claims 94-154 will be pending.

Applicant presents herein new claims 94-154 which find support throughout the specification and claims as filed, including, for example, in the specification at pages 6-8, 9-14, 17, 18, 20, 21, 24, 27, 30, 91, 92, and 94-96, Figure 4, and claim 1 as originally filed.

Applicant attaches hereto a paper and CRF version of the most recent Sequence Listing

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filed in the present application's parent.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Applicant respectfully requests that this amendment be entered without prejudice.

Applicant believes all of the claims presently before the Examiner patentably define the invention over the prior art and are otherwise in condition for ready allowance. Applicant respectfully requests early notification of the same.

Respectfully submitted,

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